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THE CONSTANCY OF BACTERIAL SPECIES IN
NORMAL FORE MILK.¹

BY H. L. BOLLEY.

It is recognized that aside from actual dirt, as, for example, drippings from the hands of the milker, dirt from his clothing, and hairs and manurial particles from the sides of the animal, that the fore milk constitutes the most productive source of the bacterial flora of milk. Schultz and others have placed quantitative determinations at from fifty to one hundred thousand per cubic centimeter. As the character of the germ content is becoming such a matter of importance in economic labors with milk and its product, it is apparent that a consideration of the types of germ present in the normal udder should command early attention of the bacteriologically inclined dairymen.

The question is of necessity, one of such breadth that it must be approached in separate phases, such, as for example, the study of the presence or absence of physiological groups, constancy of definite species, etc. During the year just closed two such points have been under investigation. The primary object, while being a matter of simple interest, had also the direct aim of determining the relation of normal fore milk to curd inflation in cheese manufactory. The results of the work have in part been reported in a paper read before the General Section of the American Association of Agricultural Colleges and Experimental Stations, July 19, 1895; showing that, in so far as the investigation had been carried, gas generating species such as are accountable for "pinhole formation" or curd inflation are not normal to the fore milk of the healthy udder.²

¹ Read before the Section of Botany of the American Association for the Advancement of Science, Springfield Meeting, August 31, 1895. Also published in *Centralblatt für Bacteriologie und Parasitenkunde*, Ab. II, B and I, No. 22-23.

² Bolley and Hall: Cheese curd inflation: Its relation to the bacterial flora of fore milk. *Centralb. f. Bact. u. Parasitenk.*, II, Ab. I, Bd., No. 22-23.

This conclusion was based upon preliminary cheese curd tests made at Madison, Wisconsin, August, 1894, and duplicated at Fargo in October, and finally upon qualitative analysis made during a period of three winter months, with ten different milch cows under consideration.

The point to be reported upon, at this time, is that of the constancy of species as found : (a) for the same cow for a given length of time ; (b) in the same teat of the same cow ; and (c) as to whether species are common to different cows or not upon same dates.

In general, the evidence of the work associated with the last named report, was to the effect that there is no evidence that germs are of any certainty common to different animals upon the same date under like conditions ; but that a certain inhabitant of the udder of the same animal may remain quite constant. Thus while only one species, number 30, was observed to be present in more than two animals of the original ten animal test upon different dates, several different species were found to occur at several dates in the same udder.

Commencing July 1st, three animals were placed under cultural investigation, number 24 of which was an animal of the original ten, also number 21. Cultures were attempted from each teat upon gelatine and agar, as often as the work could be handled, the same methods of procuring milk being used as in the previous work, except in the different tests of the same animal, the milk tube or trochar, was inserted different depths. Some sixty of these distinct milkings were taken upon fifteen different dates, during which time the cows ran upon a clean pasture during the day, being housed at night. The milk samples were taken sometimes in the morning and sometimes at night. In all, thirty-seven different species of bacteria were separated ; and, as in past work, were found to be of various physiological types, gelatine liquifiers, non-liquifiers, solid curd types, peptonizing forms, acid and alkali curdlers, etc., including bacilli, micrococci of various forms, and a streptococcus. Thus it may be said that, in general, forms collected are miscellaneous.

Results: Again, there is no marked evidence that species are common among different animals, but there is strong evidence of constancy of appearance of certain types when once present. This, perhaps, is to be expected, for it is hardly possible that in an ordinary milking all individuals could be excluded from the milk cistern and lower teat passages.

The following table and annotations may help to show the bearings of the work:

Cow No. 24	Species present, per teat, by dates.			
Teats =	No. 1	No. 2	No. 3	No. 4
*Expr. No. 1, July 2nd.	Nos. 1	Nos. 1	Nos. 1	Nos. 5
Expr. No. 2, July 3rd.	6	1	9 and 10	5, 100 & 77
Expr. No. 3, July 4th.	16	1	15	(Not taken)
Expr. No. 4, July 6th.	(Not taken)	17 and 1	20	20
Expr. No. 5, July 8th.	(Lost Cul.)	23	10, 61	26, 27, 15, 29
Expr. No. 6, July 10th.	30, 1	(Lost)	31	(Not taken)
Expr. No. 10, July 17th.	58, 53, 1	1	61	66, 20, 15, 1
Expr. No. 13, July 23rd.	96, 93, 94	1	96, 97	20, 11, 100, 1
Expr. No. 15, July 28th.	77, 67	(Not taken)	66, 100 & 67	67, 1

*The numbers in each columns—1, 2, 3 and 4 = the laboratory numbers given the different species.

Annotation No. 1, a solid curd, lactic acid forming micrococcus, is seen to be present upon every date, appearing in teat No. 2 upon all possible dates save one.

Nos. 5, 10, 15, 61 and 67 occurred twice each, the intervening days being respectively 2, 8, 7, 4 and 4. It is worthy of note that with the exception of No. 67, each of these was found each time in the same teat.

Cow No. 21	Species present, per teat, by dates.			
Teats =	No. 1	No. 2	No. 3	No. 4
Expr. No. 8, July 12th.	Nos. 45	Nos. 31	Nos. 27, 31	Nos. 20
Expr. No. 9, July, 15th.	(Lost)	31, 50	29, 53	55, 56, 57, & 31
Expr. No. 12, July 16th.	53, 51, & 56	31, 45	(Not taken)	(Lost)

Annotations:—With this animal it is to be noted that No. 31, a lactic acid forming micrococcus, is constant to all dates, and upon each date was found present in teat No. 2.

Other germs found twice each were Nos. 45, 53 and 56; but each time in a different teat.

Cow No. 26	Species present, per teat, by dates.			
Teats =	No. 1	No. 2	No. 3	No. 4
Expr. No. 7, July 1st.	33, 1	33	39, 61, 67	17, 44, 33
Expr. No. 17, July 17th.	66, 100, 67 17 and 33	33, 15	33	53, 77
Expr. No. 14, July 23rd.	33	67, 33	33	(Lost)

In these three milkings from cow No. 26, the common species to each date are seen to be Nos. 33 and 67. Out of eleven milk samples taken No. 33 occurs in the cultures nine times. The intervening dates being 16, 6 and 22 days apart. No. 33 is a streptococcus and in these distant tests, as to time separation, is a strong argument of constancy of presence being possible to an individual species. In growth characteristics this germ is almost a strict anaerobe.

Studying these tables, we find for each animal the following numbered germs present:

Cow No. 24.—Nos. 1, 5, 6, 9, 10, 100, 77, 15, 16, 17, 20, 23, 61, 26, 27, 29, 30, 31, 58, 53, 66, 96, 93, 94, 97, 11 and 67, a total of twenty-seven distinct forms.

Cow No. 21.—Nos. 45, 31, 27, 20, 50, 29, 53, 55, 56, 57 and 51, a total of eleven.

Cow No. 26.—Nos. 33, 1, 39, 61, 67, 17, 44, 66, 100, 15, 53 and 77, a total of twelve.

The forms common to three animals equal only one, No. 53, while those common to two of them are seen to be Nos. 1, 100, 77, 15, 61, 29, 31, 66, 67, 29 and 20; eleven constant forms.

General Annotations:—From these summaries it is to be noted that cow No. 24 from nine different milkings furnished twenty-seven of the thirty seven germs of the three tests, *cow*

No. 21 six and cow No. 26 four. The numbered germs from the last named animals are representative of but three milking dates each. It is thus a possibility, that further milking dates for these cows might have given others of those common to cow No. 24. While this point last named, is probably a correct consideration, it is nevertheless quite clearly indicated that the great majority of germs are but incidental in a given udder or teat to the date, perhaps, to the environments of the animal. There are, however, certain few germs found which when once present in a teat or udder, remain with marked persistence. For this capability, these are found to possess what are presumably the proper physiological functions or requirements, as for example, capability to properly thrive in or withstand the normal temperature of the animal's body, and anaerobic or semi-anaerobic faculties.

As in the case of the paper previously mentioned, this is given not as final evidence to convince upon the points mentioned or suggested, but rather as a record of preliminary work accomplished.

Again, an interesting fact is the comparatively low number of species per milk sample. In the first work, winter collections, the range was from one to four species, in this it is one to five with a rather high average number. It is also interesting, though perhaps to be expected, that quantitative determinations vary from low to high numbers for different milkings, very much in accord to these last named figures.

North Dakota Experiment Station, Fargo, N. D., August, 20, 1895.

LIFE BEFORE FOSSILS.

BY CHARLES MORRIS.

The beginning of life upon the earth is one of those mysteries which, to judge from what we now know about it, seems likely never to be solved by ascertained facts. There are mod-